Toxin Reviews
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597281

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Online Publication Date: 01 August 2008

To cite this Article Williams, W. Paul, Krakowsky, Matthew D., Windham, Gary L., Balint-Kurti, Peter, Hawkins, Leigh K. and Henry, W. Brien(2008)'IDENTIFYING MAIZE GERMPLASM WITH RESISTANCE TO AFLATOXIN ACCUMULATION',Toxin Reviews,27:3,319 — 345

To link to this Article: DOI: 10.1080/15569540802399838

URL: http://dx.doi.org/10.1080/15569540802399838

Toxin Reviews, 27:319–345, 2008

ISSN: 1556-9543 print / 1556-9551 online
DOI: 10.1080/15569540802399838

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Contamination of maize grain, Zea mays *L., with aflatoxin, a toxin produced by the fungus* Aspergillus flavus, *reduces its value and marketability. Growing hybrids with resistance is generally considered a highly desirable way to reduce* A. flavus *infection and aflatoxin accumulation. Identifying maize germplasm with resistance is critical to the development and production of such hybrids. USDA-ARS scientists at Mississippi State, Mississippi; Tifton, Georgia; and Raleigh, North Carolina; have engaged in a multilocation approach to germplasm screening. A major component of this has been the evaluation of accessions obtained from the Germplasm Enhancement of Maize (GEM) project at both Mississippi State and Tifton.*

Received 29 May 2008; revised 23 July 2008; accepted 23 July 2008.
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Selections from GEM accessions 250_01_XL370A_S11_F2S4_9214_Blk21/00-# and 2250_02_XL370A_S11_F2S4_3363_Blk03/00-# exhibited the highest levels of resistance both as lines per se and in testcrosses. Lines developed at the International Maize and Wheat Improvement Center (CIMMYT) and North Carolina State University also exhibited reduced levels of aflatoxin contamination. CML348, NC388, NC400, NC408, and NC458 were among those with low levels of aflatoxin contamination. The lines that displayed low levels of contamination should be useful in maize breeding programs for developing parental inbred lines and aflatoxin-resistant maize hybrids.

Keywords aflatoxin, *Aspergillus flavus*, maize, plant resistance, *Zea mays*

Introduction

Aflatoxin, which is produced by the fungus *Aspergillus flavus*, is the most potent carcinogen found in nature (Park and Liang, 1993; Castegnaro and McGregor, 1998; Pittet, 1998). Consumption of aflatoxin-contaminated foods is a major cause of hepatocellular carcinoma, the fifth most common cancer in humans worldwide (Wild and Hall, 2000). Although first identified and recognized as a threat after 100,000 turkeys died in England in 1961 (Detoy et al., 1971), aflatoxin is now known as a threat to other livestock and even dogs and cats (Gourma and Bullerman, 1995; Leung et al., 2006).

Aflatoxin contamination was first recognized as a major problem for maize (*Zea mays* L.) production in the southeastern United States in the 1970s. In 1977, over 90% of the maize produced in the Southeast was contaminated with aflatoxin. Aflatoxin levels exceeded the tolerance of 20 ng/g established by the U.S. Food and Drug Administration in 90% of the samples evaluated in Georgia that year (McMillian et al., 1978; Zuber et al., 1979). Maize exceeding aflatoxin levels of 20 ng/g is banned from interstate commerce.

Aflatoxin contamination has remained a chronic problem in the Southeast. In 1998, losses to aflatoxin contamination of maize in Mississippi, Louisiana, Arkansas, and Texas were estimated at $85 million (Windham and Williams, 1999, 2002). The recent increase in the use of maize as a substrate for ethanol production could exacerbate losses from aflatoxin contamination: an accumulation of aflatoxin during fermentation in spent grain

represents a serious impediment to its use in animal feeds (Lillehoj, 1978; Murthy, 2005).

Plant resistance is generally an important strategy for reducing or eliminating aflatoxin contamination in maize; however, commercial maize hybrids with adequate levels of resistance are not currently available (Widstrom et al., 1996; Windham and Williams, 1999). In response to the recognition in 1977 of the threat posed by aflatoxin contamination to maize production in the Southeast, U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) initiated research at Mississippi State, Mississippi, and Tifton, Georgia, on breeding for resistance to *A. flavus* infection and aflatoxin contamination (Widstrom, 1996; Williams, 2006). Techniques were developed for evaluating germplasm for resistance at both locations; maize germplasm with resistance to aflatoxin contamination was identified (Widstrom et al., 1984; Scott and Zummo, 1988). The release in 1988 of Mp313E, a line developed at Mississippi State University, marked the first release of maize germplasm as a source of resistance to *A. flavus* infection and aflatoxin accumulation (Scott and Zummo, 1990a). A second germplasm line, Mp420, was released in 1991 (Scott and Zummo, 1992). The release of maize germplasm population GT-MAS: gk developed at Tifton followed in 1992 (McMillian et al., 1993). Subsequently, germplasm lines Mp715 and Mp717, which were developed at Mississippi State University, were released in 1999 and 2005 (Williams and Windham, 2001, 2006). Although the germplasm lines developed at Mississippi State exhibit high levels of resistance to *A. flavus* infection and aflatoxin accumulation, they also tend to possess undesirable characteristics such as tight husk coverage and late maturity.

The evolution of the breeding program at Mississippi State was described by Williams and colleagues (2005). Although collaborations between the breeding programs at Mississippi State and Tifton have been substantial throughout the past 30 years, efforts to link these programs have increased in recent years. The goal is to develop complementary rather than duplicative programs at the two locations. The USDA-ARS Corn Host Plant Resistance Research Unit at Mississippi State and the Crop Genetics and Breeding Unit at Tifton developed shared objectives and approaches to identifying and developing maize

germplasm with resistance to *A. flavus* infection and accumulation. The USDA-ARS Plant Science Research Unit at Raleigh, North Carolina, also contributes significantly in pursuing these objectives.

The effects of environmental variation and genotype × environment interactions on *A. flavus* infection and aflatoxin accumulation complicate the process of identifying germplasm with resistance. A multilocation approach should enhance efforts to evaluate and develop maize germplasm with resistance to aflatoxin contamination. Recent evaluations identified potentially useful sources of germplasm.

Evaluation Germplasm for Resistance

A multilocation approach to identifying new sources of resistance to *A. flavus* infection and aflatoxin accumulation in maize grain permits more efficient use of available resources. New accessions are generally first evaluated in field trials with three or four replications of single-row plots of 20 plants in a randomized complete block design. Susceptible and resistant inbred lines or single crosses are included as checks within the experiments. Highly susceptible accessions are eliminated from further consideration after one year. Accessions that exhibit potentially useful levels of resistance, or the S_1 progeny of such accessions, are evaluated a second year. The most promising accessions are exchanged between locations for further evaluation and possible inclusion in each location's breeding program. These germplasm accessions are most frequently evaluated first as lines per se and subsequently in testcrosses.

Inoculation with A. flavus

At Mississippi State, the side needle technique is used for inoculating developing ears with *A. flavus* (Zummo and Scott, 1989; Windham and Williams, 2002). The top ear of each plant in a plot is inoculated approximately 7 days after mid-silk (silk emergence in 50% of the plants in a plot) with a 3.4-ml suspension containing 3×10^8 *A. flavus* conidia in sterile distilled water. The conidial suspension is injected into the side of the ear using a tree-marking gun. *A. flavus* isolate 3357, which is known to produce high levels

of aflatoxin in maize, is used as inoculum. This method usually wounds one or two kernels.

At Tifton, developing ears are inoculated approximately 20 days after mid-silk using a grafting knife dipped in a spore suspension containing 1×10^7 conidia per ml (Widstrom et al., 1981). The knife is inserted through the husk into the abaxial side of the ear. This method generally wounds 2 to 5 developing kernels. Although the side-needle technique was used at Mississippi State and the knife technique at Tifton in the experiments reported herein, experiments are currently underway at both locations to compare effects of different inoculation methods and inoculum concentrations on *A. flavus* infection and aflatoxin accumulation.

Determining Aflatoxin Levels

Inoculated ears are harvested by hand at maturity and dried in a forced-air dryer for 7 days at 38°C. After drying, the ears are shelled. The grain is thoroughly mixed using a sample splitter and ground using a Romer mill (Union, Missouri). Aflatoxin contamination in a 50-g subsample is then determined using the Vicam Aflatest protocol (Vicam, Watertown, Massachusetts).

Statistical Analysis

Data were analyzed using the SAS general linear models procedure (SAS Institute, 2003). Means were compared using Fisher's Protected Least Significant Difference (LSD) at $P = 0.05$ (Steel and Torrie, 1980). Because the error variances on the original scale were heterogeneous and tests of significance in the analysis of variance require that experimental errors be independently and normally distributed, values for aflatoxin contamination were transformed prior to analysis of variance by adding 1 and taking the logarithm of each number ($\ln[Y + 1]$). The logarithmic transformation is appropriate for positive integers that cover a wide range, but it cannot be used directly for values of zero. Each number was increased by 1 prior to taking logarithms: $\ln(Y + 1)$ behaves like the square root transformation for numbers less than 10 and differs little from $\ln Y$ for larger values. Means obtained

by transforming back to the original scale using antilogarithms of means of the ln Y values are geometric means of the original data.

Identifying New Sources of Resistance for Breeding

In 2007, progenies of accessions originally obtained through the Germplasm Enhancement of Maize (GEM) project in 2003 were evaluated at the R. R. Foil Plant Science Research Center at Mississippi State University following procedures previously described. Although germplasm representing multiple sources were evaluated in 2003, most of the accessions proved to be susceptible to aflatoxin contamination in 2003 or subsequent years. Lines selected from two accessions, 2250_02_XL370A_S11_ 2S4_3363_Blk03/00-# and 2250_01_XL370 AS11-F2S4_9214_Blk21/00-#, have, however, exhibited levels of resistance that equal or exceed those of the best resistant checks through four or five generations of self-pollination (Tables 1, 2). These selections were among the lines evaluated at Tifton in 2007, and some of them exhibited the lowest levels of aflatoxin accumulation in the trial (Table 3). Selections made at Tifton from related germplasm also exhibited useful levels of resistance. Selections at Tifton from other germplasm sources also exhibited potentially useful levels of resistance: [SCRp3N14F2S3 (2410-003/99)]-1, Cuba117:S15-101-1-B-B-B-2-1, and Cuba164:S1517-163-1-B-B-B-4. Cuba117:S15-101-1-B-B-B-2-1 and related lines selected for resistance at Tifton also exhibited resistance in an evaluation at Mississippi State in 2007 (Table 4). Again, (2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-1-7-2-B and related lines, whether initially evaluated at Tifton or Mississippi State, exhibited resistance. Included in this trial were also newly obtained GEM lines that had not been previously evaluated for resistance to *A. flavus* infection or aflatoxin accumulation, but rather had been selected for resistance to *Fusarium verticillioides*. Selections from 1883-001/98_DKXL370AN11F2S3_7521-05 share common parentage, DKXL370A, with lines selected from 2250-01_XL370A_S11_F2S4_9214_Blk21/00-#. The latter are in a stiff stalk background and the former are in a nonstiff stalk background. Aflatoxin contamination was relatively high in lines such as BR51721:N2012-397-001-B-B, PE001n16F2S2-239-B-B-B,

TABLE 1 Mean Aflatoxin Accumulation in Genotypes Selected from GEM Accessions and Evaluated at Mississippi State in 2007

	Aflatoxin[a]	
Genotype	ng/g[b]	ln(y + 1)
SC212M[s]	3710	8.22
GA209[s]	3282	8.10
Va35[s]	2544	7.84
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-1-1-B	655	6.49
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-1-3-B	306	5.73
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-14-1-B	304	5.72
Mp717[r]	253	5.54
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-9-2-B	235	5.47
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-4-3-3-B	184	5.22
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-4-3-2-B	151	5.03
Mp313E[r]	146	5.00
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-4-1-1-B	128	4.87
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-4-3-1-B	119	4.79
Mp04:97	119	4.79
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-9-1-B	114	4.75
NC 400	110	4.71
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-7-B-B	71	4.29
NC388	41	3.75
(2250-02_XL370A_S11_F2S4_3363_Blk03/00-#)-6-1-2-B	38	3.68
Mean		5.47
LSD (0.05)		1.94
CV		20

[a]Data were transformed (ln[y + 1]) before statistical analysis.
[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).
[s]Susceptible check.
[r]Resistant check.

and selections from AR16026:S17. These do not appear likely to be useful sources of resistance.

In top crosses, several of the new selections from GEM exhibited significantly higher levels of resistance than the susceptible checks (Table 5). Only one, 1886-003/98 DKXL370AN11F2S3 × T8, did not differ significantly from the resistant check, Mp313E × Mo18W. Selections from Cuba117:S15 performed well in crosses with lines developed at Mississippi State. Aflatoxin contamination was significantly lower in Mp313E × Mp717, a cross between two

TABLE 2 Mean Aflatoxin Accumulation in Genotypes Selected from GEM Accessions and Evaluated after Inoculation with *A. flavus* at Mississippi State in 2007

	Aflatoxin[a]	
Genotype	ng/g[b]	ln(y + 1)
GA209[s]	3451	8.15
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-11-1-1-B	1085	6.99
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-6-1-1-B	585	6.37
Mp717[r]	526	6.27
Mp313E[r]	473	6.16
Mp715[r]	294	5.69
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-10-1-1-B	170	5.14
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-4-1-1-B	157	5.07
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-5-1-1-B	118	4.78
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-4-1-1-B	80	4.40
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-1-1-1-B	67	4.23
Mp04:97	48	3.90
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-4-1-1-B	46	3.85
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-10-1-1-B	41	3.76
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-3-1-1-B	35	3.59
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-7-1-1-B	26	3.31
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-3-1-1-B	20	3.08
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-1-1-1-B	17	2.91
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-9-1-1-B	16	2.86
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-2-B-1-B	10	2.41
Mean		4.65
LSD (0.05)		1.34
CV		20

[a]Data were transformed [ln(y + 1)] before statistical analysis.
[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).
[s]Susceptible check.
[r]Resistant check.

lines released from Mississippi State, than in all other crosses (Williams and Windham, 2001, 2006).

Crosses between a group of lines developed at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico and LH311 were evaluated at Tifton in 2007 (Table 6). Aflatoxin accumulation was lower in crosses with several CIMMYT lines including CML373, CML320, CML316, and CML483 than in the check, Mo18W × Mp313E. Aflatoxin contamination was

TABLE 3 Mean Aflatoxin Accumulation in Genotypes Selected at Mississippi State, Mississippi, or Tifton, Georgia, and Evaluated at Tifton in 2007

Genotype	Selection Site	Aflatoxin (ng/g)[b]	Aflatoxin ln(y + 1)
(CHS775N19F1S3 (H6141-11/96))-1	GA	1635[b]	7.40
(2253-01_XL370A_S11_F2S4_9220-Blk24/00-sib)-1	GA	1587	7.37
(CHS775N19F1S3 (H6141-11/96))-2	GA	1379	7.23
(DK888N11F2S3 (9353-01/97))-2	GA	1352	7.21
DK212T N11 F2S2 7431-3-3[3050-003/00]1-B-1	GA	1299	7.17
AR03056:N09-182-1-B-B-B-2	GA	1064	6.97
DK888 N11 F2S2 7451-3-1[3291-001/00]1-1	GA	1032	6.94
GA209[s]		828	6.72
(CHS775N19F1S3 (2405-010/99))-1	GA	811	6.70
T173[s]		811	6.70
Pas14 S21 F1S3 C608-1-5-B-1	GA	787	6.67
(DKXL370AN11F2S3 (1895-001/98)-1	GA	772	6.65
GEMS-0033-B-1	GA	749	6.62
DK212T N11 F2S2 7431-3-3[3050-003/00]1-B-2	GA	727	6.59
DK212T N11 F1S3 2326b-14-394-B-1	GA	727	6.59
AR03056:N09-191-1-B-B-B-1	GA	678	6.52
DK212T N11 F2S2 7431-3-53[3315-003/00]1-B-1	GA	625	6.44
CUBA117:S15-101-1-B-B-B-4-1	GA	613	6.42
(DK888N11F2S3 (9353-01/97))-1	GA	595	6.39
GEMS-0033-B-2	GA	595	6.39
GEMS-0012-B-2	GA	689	6.38
AR03056:N09-182-1-B-B-B-1	GA	583	6.37
DK888 N11 F2S2 7451-3-1[3291–001/00]1-2	GA	583	6.37
Pas14 S21 F1S3 C608-1-5-B-2	GA	571	6.35
DK212T N11 F2S3 7431-03-3-B-1	GA	538	6.29
(DKXL370AN11F2S3 (1881-006/98)-1	GA	527	6.27
DK888 N11 F2S2 7451-3-1[3291-001/00]1-3	GA	522	6.26
GEMS-0022-B-1	GA	512	6.24
GEMS-0012-B-1	GA	507	6.23
DK888 N11 F1S3 2141-2-34-B-1	GA	482	6.18
DK212T N11 F1S3 2326b-14-394-B-2	GA	463	6.14
PE001n16F2S2-181-B-1	GA	458	6.13
(CHS775N19F1S3 (2405-010/99))-2	GA	436	6.08
(DK212T N11 F2S2 7431-3-53[3315-003/00]1-2	GA	419	6.04
GEMS-0022-B-2	GA	406	6.01
AR16026:S17-10-1-B-B-B-B-3	GA	368	5.91
DK212T N11 F2S2 7431-3-22[3315-022/00]1-B-1	GA	364	5.90
AR16026:S17-10-1-B-B-B-B-1	GA	350	5.86
(DK212TN11F2S3 (1507-001/98))-2	GA	350	5.86
(2250_01_XL370AS11-F254_9214_Blk21/00-#)-1-2-B-1-B	MS	346	5.85

(Continued on next page)

TABLE 3 Mean Aflatoxin Accumulation in Genotypes Selected at Mississippi State, Mississippi, or Tifton, Georgia, and Evaluated at Tifton in 2007 (*Continued*)

Genotype	Selection Site	Aflatoxin (ng/g)[b]	Aflatoxin ln(y + 1)
(DK888S11F2S3 (1415-01/97))-2	GA	346	5.85
(DKXL380N11F2S3 (2423-017/99))-1	GA	316	5.76
(DKXL370AN11F2S3 (1881-002/98))-1	GA	313	5.75
(CHS775N19F1S3 (1312-01/97))-1	GA	310	5.74
AR16026:S17-10-1-B-B-B-B-2	GA	292	5.68
DK212T N11 F2S3 7431-03-3-B-2	GA	292	5.68
(DK212TN11F2S3 (1507-001/98))-1	GA	286	5.66
(DK212T N11 F2S2 7431-3-53[3315-003/00]1-1	GA	280	5.64
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-6-1-1-B	MS	280	5.64
DK212T N11 F2S2 7431–3-22[3315-022/00]1-B-2	GA	280	5.64
(2283–01_XL380_S11_F2S4_9229-Blk20/00-sib)-2	GA	275	5.62
AR16026:S17-10-1-B-B-B-B-4	GA	267	5.59
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-11-1-1-B	MS	264	5.58
(DKXL380N11F2S3 (2423–017/99))-2	GA	261	5.57
(DKXL370AN11F2S3 (1886-003/98)-1	GA	259	5.56
Cuba117:S15-101-1-B-B-B-1-1	GA	249	5.52
(DK888S11F2S3 (1415-01/97))-1	GA	246	5.51
(2283-01_XL380_S11_F2S4_9229-Blk20/00-sib)-1	GA	232	5.45
(2258-03_XL380_S11_F2S4_71/97_Bulk/98-sib)-3	GA	220	5.40
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-4-1-1-B	MS	220	5.40
(DK888S11F2S3 (1415-06/97))-1	GA	218	5.39
Cuba164:S1517-163-1-B-B-B-1	GA	210	5.35
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-10-1-1-B	MS	203	5.32
Cuba117:S15-101-1-B-B-B-5-1	GA	203	5.32
(DKXL370AN11F2S3 (1883-001/98)-1	GA	203	5.32
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-3-1-1-B	MS	201	5.31
(DKXL370AN11F2S3 (1883-002/98)-1	GA	190	5.25
(2282-01_XL380_S11_F2S4_9226-Blk26/00-sib)-1?	GA	190	5.25
(2086-01_DK212T_S11_F2S4_9154-Blk20/00-sib)-3	GA	190	5.25
DXKL370:N11a20-322-1-B-B-2-1	GA	190	5.25
Cuba164:S1517-163-1-B-B-B-3	GA	182	5.21
(2258–03_XL380_S11_F2S4_71/97_Bulk/98-sib)-1	GA	175	5.17
(DK888S11F2S3 (1415-06/97))-2	GA	173	5.16
(2250-01_XL370A_S11_F2S4_9214-Blk21/00-sib)-2	GA	171	5.15
DK888 N11 F1S3 2141-2-34-B-2	GA	170	5.14

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TABLE 3 Mean Aflatoxin Accumulation in Genotypes Selected at Mississippi State, Mississippi, or Tifton, Georgia, and Evaluated at Tifton in 2007

Genotype	Selection Site	Aflatoxin (ng/g)[b]	Aflatoxin ln(y + 1)
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-3-1-1-B	MS	168	5.13
Cuba164:S1517-163-1-B-B-B-2	GA	161	5.09
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-1-1-1-B	MS	149	5.01
Cuba164:S1517-163-1-B-B-B-4	GA	148	5.00
Mp717[r]	MS	148	5.00
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-4-1-1-B	MS	148	5.00
Cuba117:S15-101-1-B-B-B-2-1	GA	133	4.90
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-5-1-1-B	MS	131	4.88
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-7-1-1-B	MS	127	4.85
DXKL370:N11a20-322-1-B-B-2-2	GA	125	4.83
(2258-03_XL380_S11_F2S4_71/97_Bulk/98-sib)-2	GA	122	4.81
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-4-1-1-B	MS	117	4.77
(2086–01_DK212T_S11_F2S4_9154-Blk20/00-sib)-2	GA	115	4.75
(2250-02_XL370A_S11_F2S4_3363-Blk03/00-sib)-1	GA	111	4.72
(2086-01_DK212T_S11_F2S4_9154-Blk20/00-sib)-1	GA	110	4.71
(2250_01_XL370AS11-F2S54_9214_Blk21/00-#)-1-10-1-1-B	MS	110	4.71
Mp04:97	MS	108	4.69
SCRGp3N14F2S3 (2410-003/99)-1	GA	107	4.67
Mp313E[r]	MS	82	4.42
(2250-02_XL370A_S11_F2S4_3363-Blk03/00-sib)-2	GA	82	4.42
(2250-01_XL370A_S11_F2S4_9214-Blk21/00-sib)-1	GA	78	4.37
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-9-1-1-B	MS	70	4.27
(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-1-1-1-B	MS	70	4.26
Mean			5.69
LSD (0.05)			0.93
CV			8

[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).

[s]Susceptible check.

[r]Resistant check.

TABLE 4 Mean Aflatoxin Accumulation in Genotypes Selected at Mississippi State, Mississippi, Tifton, Georgia, or Obtained through GEM and Evaluated for Aflatoxin Accumulation after Inoculation with *A. flavus* at Mississippi State in 2007

		Aflatoxin[a]	
Genotype	Source	ng/g[b]	ln (y + 1)
GA209[s]		9652	9.17
SC212M[s]		7206	8.88
(2258-03_XL380_S11_F2S4_71/97_Bulk/98-sib)-3	GA	2715	7.91
Va35[s]		2551	7.84
BR51721:N2012–397-001-B-B	GEM	2461	7.81
(2258-03_XL380_S11_F2S4_71/97_Bulk/98-sib)-1	GA	2254	7.72
AR16026:S17-10-1-B-B-B-3	GA	1918	7.56
AR16026:S17-10-1-B-B-B-1	GA	1683	7.43
(2258–03_XL380_S11_F2S4_71/97_Bulk/98-sib)-2	GA	1337	7.20
(1895-001/98_DKXL370AN11F2S3_7521-29)-B-B	GEM	1317	7.18
AR16026:S17-237-002-B-B-B-B-B	GEM	994	6.90
(1507-001/98_DK212TN11F2S3_7431-03)-B-B	GEM	808	6.70
PE001n16F2S2-239-B-B-B	GEM	703	6.56
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-4-11-2-B	MS	702	6.55
Mp420[r]		690	6.54
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-1-6-1-B	MS	642	6.47
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-1-4-1-B	MS	589	6.38
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-4-11-1-B	MS	482	6.18
(2086-01_DK212T_S11_F2S4_9154-Blk20/00-sib)-2	GA	377	5.93
Cuba117:S15-101-1-B-B-B-1-1	GA	332	5.81
(2086-01_DK212T_S11_F2S4_9154-Blk20/00-sib)-1	GA	330	5.80
(1883-002/98_DKXL370AN11F2S3_7521-05)-B-B	GEM	324	5.78
DKXL370:N11a20-322-001-B-B-Sib-B	GEM	258	5.56
(1881-002/98_DKXL370AN11F2S3_7521-05)-B-B	GEM	255	5.54
AR16026:S17-10-1-B-B	GEM	234	5.46
(1886-003/98_DKXL370AN11F2S3_7521-05)-B	GEM	232	5.46
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-1-9-1-B	MS	223	5.41
Mp717[r]		215	5.38
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-4-9-3-B	MS	191	5.26
NC300		189	5.25
Cuba117:S15-101-1-B-B-B-5-1	GA	136	4.92
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-5-1-2-B	MS	98	4.60

(Continued on next page)

TABLE 4 Mean Aflatoxin Accumulation in Genotypes Selected at Mississippi State, Mississippi, Tifton, Georgia, or Obtained through GEM and Evaluated for Aflatoxin Accumulation after Inoculation with *A. flavus* at Mississippi State in 2007 (*Continued*)

		Aflatoxin[a]	
Genotype	Source	ng/g[b]	ln (y + 1)
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-4-4-1-B	MS	93	4.54
Mp313E[r]		91	4.53
(1883-001/98_DKXL370AN11F2S3_7521-05)-B-B	GEM	83	4.43
Cuba117:S15-101-1-B-B-B-2-1	GA	68	4.23
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-5-1-3-B	MS	67	4.22
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-5-4-2-B	MS	67	4.22
(2250-02_XL370A_S11_F2S4_3363-Blk03/00-sib)-1	GA	35	3.58
(2250-01_XL370A_S11_F2S4_9214_Blk21/00-#)-1-7-2-B	MS	29	3.42
Mean			5.95
LSD (0.05)			1.17
CV			13

[a]Data were transformed [ln(y+1)] before statistical analysis.
[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).
[s]Susceptible check.
[r]Resistant check.

lowest in the cross, LH195 × Tx772. Tx772 was developed and released in Texas as a source of resistance to aflatoxin contamination (Llorente et al., 2004).

In 2007, crosses between several of the selections evaluated at Mississippi State and listed in Table 2 and Va35 were evaluated for aflatoxin contamination at three locations: Mississippi State, Mississippi; Tifton, Georgia; and Raleigh, North Carolina. In the field trial at Tifton, ears were inoculated 20 days after mid-silk using a knife dipped in an *A. flavus* spore suspension. The side-needle technique was used at the other two locations; ears were inoculated 7 days after mid-silk. Aflatoxin contamination was somewhat greater at Tifton than at the other two locations. Levels of contamination at Mississippi State and Raleigh were generally consistent for the testcrosses (Table 7). The fact that

TABLE 5 Mean Aflatoxin Accumulation in Topcrosses of Lines Selected at Mississippi State, Mississippi, or Obtained through GEM and Evaluated for Aflatoxin Accumulation after Inoculation with *A. flavus* at Mississippi State in 2007

		Aflatoxin[a]	
Genotype	Source	ng/g[b]	ln (y + 1)
T173 × Va35[s]		1141	7.04
1507-001/98 DK212TN11F2S3xT8	GEM	915	6.82
GA209 × SC212m[s]		631	6.45
BR51721:N2012-397-001 × HC33	GEM	593	6.39
1895-001/98 DKXL 370AN11F2S3xT8	GEM	548	6.31
T173 × LH219[s]		442	6.09
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5] × LH210	GEM	422	6.05
S2B73 × Mp04:97	MS	373	5.92
1883-002/98 DKXL 370AN11F2S3xT1	GEM	306	5.73
PE001n16F2S2-239 × LH200	GEM	273	5.61
Mp 04:104 × B110	MS	270	5.60
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-11-1-1] × Va35	MS	258	5.56
S2B73 × Mp04:96	MS	238	5.48
Mp 04:96 × LH210	MS	213	5.36
AR16026:S17-237-002-B-B-B-B × DKXL370:N11a20-322-001-B-B-B-B	GEM	197	5.29
1881-002/98 DKXL 370AN11F2S3xT1	GEM	192	5.26
B110 × Mp04:96	MS	172	5.15
AR16026:S17-10-1-B × LH283	GEM	169	5.13
S2B73 × Mp04:110	MS	158	5.07
1883-001/98 DKXL 370AN11F2S3xT8	GEM	143	4.97
(Cuba117:S15)F7-1a-1-1-1-1 × Mp04:110	MS	139	4.94
(Cuba117:S15)F7-1a-1-1-1-1 × Mp04:96	MS	116	4.76
B110 × Mp04:110	MS	103	4.65
Mp 04:97 × Mo18W	MS	102	4.63
1886-003/98 DKXL 370AN11F2S3xT8	GEM	100	4.61
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-1-1-1] × Va35	MS	94	4.56
(CUBA117:S15)F7-1a-1-1-1-1 × Mp04:97	MS	80	4.40
B110 × Mp04:97	MS	65	4.20
Mp04:97 × Va35	MS	52	3.97
Mp04:97 × NC388	MS	40	3.72
Mp313E × Mo18W[r]		40	3.72
Mp04:97 × LH210	MS	31	3.45
Mp313E × Mp717[r]		7	2.05
Mean			5.40
LSD (0.05)			1.14
CV			15

TABLE 6 Mean Aflatoxin Accumulation in Test Crosses and Evaluated after Inoculation with *A. flavus* at Tifton, Georgia, in 2007

	Aflatoxin[a]	
Genotype	ng/g[b]	ln(y + 1)
LH311 × CML321	992	6.90
GEMS-0033 × LH195	772	6.65
LH311 × CML336	749	6.62
LH311 × CML377	727	6.59
CML335 × LH311	671	6.51
LH311 × CML315	632	6.45
SC212M × GA209	625	6.44
LH311 × CML312	613	6.42
CML325 × LH311	607	6.41
LH210 × LH311	607	6.41
LH311 × CML422	527	6.27
CML337 × LH311	518	6.25
LH311 × CML385	487	6.19
CML387 × LH311	482	6.18
LH311 × P2	482	6.18
CML112 × LH311	458	6.13
LH311 × CML486	445	6.10
LH311 × CML176	432	6.07
LH311 × CML493	427	6.06
LH311 × CML334	419	6.04
LH311 × CL-RCY016	415	6.03
LH311 × CML484	411	6.02
LH311 × CY-1	411	6.02
LH311 × A1-1	402	6.00
P27 × LH311	402	6.00
LH311 × Mp04:117	398	5.99
CML331 × LH311	387	5.96
LH311 × CML328	379	5.94
LH311 × CML480	368	5.91
LH311 × CML424	364	5.90
CML369 × LH311	357	5.88
LH311 × P56	353	5.87
LH311 × CML485	343	5.84
LH311 × CML375	336	5.82
LH311 × CML318	333	5.81
LH311 × Mp04:127	329	5.80
T173 × Va35	329	5.80
CML378 × LH311	329	5.80

(Continued on next page)

TABLE 6 Mean Aflatoxin Accumulation in Test Crosses and Evaluated after Inoculation with *A. flavus* at Tifton, Georgia, in 2007 (*Continued*)

	Aflatoxin[a]	
Genotype	ng/g[b]	ln(y + 1)
LH311 × CML333	326	5.79
LH311 × CML330	323	5.78
Mp04:96 × LH311	323	5.78
LH311 × CML326	307	5.73
LH311 × CML386	307	5.73
LH311 × CML368	304	5.72
LH311 × CML327	301	5.71
LH311 × CML421	298	5.70
LH311 × CML332	295	5.69
LH311 × Mp313E	278	5.63
LH311 × CML285	278	5.63
LH311 × CML487	278	5.63
LH311 × CML338	278	5.68
LH311 × CML374	275	5.62
LH311 × P50	269	5.60
Mp717 × Mp313E	261	5.57
LH311 × CML367	259	5.56
CML376 × LH311	259	5.56
LH311 × Mp04:119	244	5.50
CML370 × LH311	236	5.47
LH311 × CML371	225	5.42
LH311 × CML317	218	5.39
LH311 × CML372	218	5.39
LH311 × CML287	209	5.35
LH311 × CML313	201	5.31
CML416 × LH311	199	5.30
Mo18W × Mp313E	191	5.26
LH311 × Mp95:512	188	5.24
LH311 × CML483	188	5.24
LH311 × CML316	158	5.07
LH311 × CML320	152	5.03
LH311 × CML373	146	4.99
CY-2 × LH311	127	4.85
LH195 × Tx772	110	4.71
Mean		5.82
LSD (0.05)		0.79
CV		7

[a]Data were transformed (ln[y + 1]) before statistical analysis.

[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).

TABLE 7 Mean Aflatoxin Accumulation in Testcrosses of Lines Selected at Mississippi State and Evaluated at Raleigh, NC; Tifton, Georgia; and Mississippi State, Mississippi in 2007

	Aflatoxin[a]							
	Raleigh		Tifton		Mississippi State		Overall	
Genotype	ln(y + 1)	ng/g[b]	ln(y + 1)	ng/g[b]	ln(y + 1)	ng/g[b]	ln(y + 1)	ng/g[b]
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-4-1-1] × Va35	4.11	60	5.18	177	4.02	55	4.44	84
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-10-1-1] × Va35	4.43	83	5.27	193	3.81	44	4.50	89
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-11-1-1] × Va35	6.23	507	4.98	144	5.34	207	5.52	247
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-1-1-1] × Va35	3.67	38	5.18	176	4.27	71	4.37	78
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-3-1-1] × Va35	4.31	73	4.33	75	3.85	46	4.16	63
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-5-4-1-1] × Va35	5.45	232	4.85	127	5.14	170	5.15	171
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-1-1-1] × Va35	3.38	28	5.06	156	3.90	49	4.11	60
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-2-B-1] × Va35	4.54	93	4.95	140	4.70	109	4.73	112
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-3-1-1] × Va35	4.98	145	4.99	145	3.77	42	4.58	96
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-4-1-1] × Va35	6.41	608	5.41	224	5.72	305	5.85	346

(Continued on next page)

TABLE 7 Mean Aflatoxin Accumulation in Testcrosses of Lines Selected at Mississippi State and Evaluated at Raleigh, NC; Tifton, Georgia; and Mississippi State, Mississippi in 2007 (*Continued*)

	Aflatoxin[a]							
	Raleigh		Tifton		Mississippi State		Overall	
Genotype	ln(y + 1)	ng/g[b]	ln(y + 1)	ng/g[b]	ln(y + 1)	ng/g[b]	ln(y + 1)	ng/g[b]
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-5-1-1] × Va35	4.31	73	4.07	58	4.71	111	4.37	78
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-6-1-1] × Va35	6.22	500	6.01	406	6.16	473	6.13	458
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-7-1-1] × Va35	4.65	103	5.32	203	4.34	76	4.77	116
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-8-1-1] × Va35	2.83	16	5.07	158	3.70	40	3.87	47
[(2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-9-1-1] × Va35	4.77	117	4.77	117	4.53	92	4.69	108
Mp04:97 × Va35	6.46	641	4.70	109	3.99	53	5.05	155
Mp313E × Va35	6.25	517	4.95	141	3.29	26	4.83	124
Mp715 × Va35	6.21	495	5.62	276	5.51	247	5.78	323
Mp717 × Va35	5.69	294	5.32	203	4.90	133	5.30	200
GA209 × Va35	7.13	1242	5.97	390	5.90	365	6.33	561
Mean	5.10		5.10		4.58		4.93	
LSD(0.05)	1.50		0.97		1.27		0.72	
CV	21		13		20		18	

[a]Data were transformed (ln[y+1]) before statistical analysis.
[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).

the same method of inoculation was used at those two locations may have contributed to the similarities of results. The two selections, (2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-4-11-1-1-B and (2250_01_XL370AS11-F2S4_9214_Blk21/00-#)-1-6-1-1-B, that had the highest levels of contamination as lines (Table 2) also had high levels of contamination as testcrosses. The lines that sustained lower levels of contamination as lines per se also exhibited less contamination in testcrosses.

Breeding for Resistance

Line development based on S_1 progeny performance has been the most frequently used approach in developing germplasm lines with resistance to aflatoxin contamination at Mississippi State. The germplasm lines that have been developed and released as sources of resistance to *A. flavus* infection and aflatoxin contamination are late maturing and also lack other desirable agronomic qualities (Scott and Zummo, 1990a, 1992; McMillian et al., 1993; Williams and Windham, 2006). To develop lines that will be more useful in applied breeding programs, it is desirable to develop lines that are not only resistant to aflatoxin contamination but also have other desirable agronomic qualities. This generally involves making crosses between germplasm that has resistance to aflatoxin contamination and lines that have desirable agronomic qualities.

Identifying Resistance in Released Lines

Although the search for new sources of resistance to *A. flavus* at both Tifton and Mississippi State focuses primarily on the evaluation of new accessions obtained through the GEM project, it is also important to determine whether released inbred and germplasm lines from other sources exhibit resistance. Many of the recent releases from North Carolina State University have been screened for resistance (Widstrom et al., 1984). A few lines, including NC388, NC400, NC408, and NC458 exhibited moderate resistance in these evaluations. NC388, NC400, and NC458 were selected from tropical germplasm. NC408 was developed from the cross SC76 × B52, and SC76 had been earlier identified as having moderate resistance to *A. flavus* kernel infection. NC408

TABLE 8 Mean Aflatoxin Accumulation in Inbred Lines Evaluated at Mississippi State in 2007

	Aflatoxin[a]	
Genotype	ng/g[b]	ln (y + 1)
SC212M[s]	9803	9.19
GA209[s]	7376	8.91
Tx114	2660	7.89
Tx805	2167	7.68
Mo17	1530	7.33
Mp716	1345	7.20
NC37	1142	7.04
B73	1110	7.01
Va35	891	6.79
CML343	875	6.77
T173	769	6.65
MI82	662	6.50
CML247	644	6.46
Va58	633	6.45
Mp496	547	6.31
Mp420	521	6.26
Mp707	507	6.23
Mo18W	494	6.20
Mp717[r]	489	6.19
NC300	473	6.16
Mp708	440	6.09
Tex6	428	6.06
Mp76:213	415	6.03
Mp313E[r]	397	5.99
NC400	339	5.83
CML342	335	5.82
Mp71:231	303	5.72
Va99	295	5.69
Tx601	294	5.69
CML341	260	5.56
Mp04:96	233	5.45
NC388	224	5.41
NC408	216	5.38
NC458	208	5.34
Mp715[r]	195	5.28
Mp04:112	164	5.11
Mp04:97	158	5.07

(Continued on next page)

TABLE 8 Mean Aflatoxin Accumulation in Inbred Lines Evaluated at Mississippi State in 2007 (*Continued*)

	Aflatoxin[a]	
Genotype	ng/g[b]	ln (y+1)
T115	116	4.76
Mp494	93	4.54
CML348	21	3.08
Mean		6.22
LSD (0.05)		1.21
CV		12

[a]Data were transformed (ln[y + 1]) before statistical analysis.
[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).
[s]Susceptible check.
[r]Resistant check.

exhibited significant general combining ability for aflatoxin resistance in a diallel cross (Scott and Zummo, 1990b).

In 2007, a group of inbred lines were evaluated at Mississippi State (Table 8). A line developed at CIMMYT, CML348, had the lowest value for accumulation of aflatoxin. Aflatoxin contamination was also low in Mp494, an unreleased line developed in Mississippi, and T115, a line developed in Tennessee. NC388, NC408, and NC458 did not differ from the best-performing resistant check, Mp715. Mp04:96 and Mp04:97, selections from the cross Mp715 × Va35, and Mp04:112, a selection from Mp313E × Va35, exhibited levels of resistance equivalent to that of Mp715.

In an evaluation of crosses among these lines, the levels of aflatoxin contamination were relatively low (Table 9). Aflatoxin contamination was highest in two susceptible checks, GA209 × SC212m and SC212m × SC229. In crosses between NC388 and lines selected for resistance, aflatoxin levels were generally low. Mp715 × NC388, Mp04:97 × NC388, Mp494 × Mp717, Mp715 × NC400, Mp313E × Mp717, and Mp717 × NC388 were among the crosses with low levels of aflatoxin accumulation although the ears were inoculated with *A. flavus*.

Results of the evaluation of lines developed from the crosses Mp715 × Va35, Mp 715 × T171, and Mp313E × Va35 are given in Table 10. Among the selections from Mp715 × Va35, Mp04:96 and

TABLE 9 Mean Aflatoxin Accumulation in Single Crosses Inoculated Using the Side-Needle Technique and Evaluated at Mississippi State in 2007

	Aflatoxin[a]	
Single Cross	ng/g[b]	ln (y + 1)
GA209 × SC212M	591	6.38
SC212M × SC229	450	6.11
Mp04:86 × NC388	332	5.81
Mp313E × Mp494	291	5.68
Mp313E × Mo18W	248	5.52
Mp04:86 × NC400	201	5.31
GA209 × Va35	149	5.01
Mp04:119 × NC400	100	4.62
Mp717 × NC400	89	4.50
Mp04:96 × NC388	87	4.47
Mp715 × Mp717	52	3.96
Mp04:110 × NC400	47	3.88
Mp04:126 × NC388	38	3.66
Mp04:127 × NC388	39	3.69
Mp313E × NC400	35	3.57
Mp494 × Mp715	35	3.59
Mp04:120 × NC388	34	3.54
Mp04:127 × NC400	30	3.43
Mp04:119 × NC388	23	3.19
Mp04:96 × NC388	20	3.03
Mp313E × NC388	12	2.54
Mp313E × Mp715	10	2.43
Mp313E × Mp708	9	2.27
Mp717 × NC388	9	2.25
Mp313E × Mp717	7	2.08
Mp715 × NC400	6	1.99
Mp494 × Mp717	5	1.79
Mp04:97 × NC388	4	1.63
Mp715 × NC388	1	0.96
Mean		3.69
LSD (0.05)		1.92
CV		32

[a]Data were transformed (ln[y + 1]) before statistical analysis.

[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).

Mp04:97 had the lowest levels of aflatoxin contamination. The level of contamination for Mp04:97 was significantly lower than for the resistant parent, Mp715. This line also performed well

TABLE 10 Mean Aflatoxin Accumulation in Advanced Breeding Lines Inoculated with *A. flavus* Using the Side-Needle Technique at Mississippi State in 2007

		Aflatoxin[a]	
Genotype	Source Population	ng/g[b]	ln(y+1)
Mp04:85	Mp715 × Va35	3118	8.05
Mp04:86	Mp715 × Va35	897	6.80
Mp04:87	Mp715 × Va35	609	6.41
Mp04:88	Mp715 × Va35	1492	7.31
Mp04:89	Mp715 × Va35	2139	7.67
Mp04:90	Mp715 × Va35	1068	6.97
Mp04:91	Mp715 × Va35	563	6.33
Mp04:92	Mp715 × Va35	1079	6.99
Mp04:93	Mp715 × Va35	289	5.66
Mp04:94	Mp715 × Va35	479	6.18
Mp04:95	Mp715 × Va35	466	6.15
Mp04:96	Mp715 × Va35	130	4.88
Mp04:97	Mp715 × Va35	11	2.54
Mp04:98	Mp715 × Va35	779	6.66
Mp04:99	Mp715 × Va35	825	6.72
Mp04:100	Mp715 × Va35	634	6.45
Mp04:101	Mp715 × Va35	930	6.84
Mp04:102	Mp715 × Va35	539	6.29
Mp04:103	Mp715 × Va35	747	6.62
Mp04:104	Mp715 × Va35	592	6.39
Mp04:105	Mp715 × Va35	559	6.33
Mp04:106	Mp715 × TI71	276	5.63
Mp04:107	Mp715 × TI71	71	4.28
Mp04:108	Mp313E × Va35	260	5.67
Mp04:109	Mp313E × Va35	131	4.88
Mp04:110	Mp313E × Va35	21	3.12
Mp04:111	Mp313E × Va35	126	4.85
Mp04:112	Mp313E × Va35	134	4.91
Mp04:113	Mp313E × Va35	120	4.80
Mp04:114	Mp313E × Va35	76	4.35
Mp04:115	Mp313E × Va35	52	3.99
Mp04:116	Mp313E × Va35	158	5.07
Mp04:117	Mp313E × Va35	198	5.29
Mp04:118	Mp313E × Va35	286	5.66
Mp04:119	Mp313E × Va35	281	5.64
Mp04:120	Mp313E × Va35	412	6.02
Mp04:121	Mp313E × Va35	182	5.21
Mp04:122	Mp313E × Va35	164	5.11

(Continued on next page)

TABLE 10 Mean Aflatoxin Accumulation in Advanced Breeding Lines Inoculated with *A. flavus* Using the Side-Needle Technique at Mississippi State in 2007 (*Continued*)

Genotype	Source Population	Aflatoxin[a] ng/g[b]	Aflatoxin[a] ln(y+1)
Mp04:123	Mp313E × Va35	232	5.45
Mp04:124	Mp313E × Va35	259	5.56
Mp04:125	Mp313E × Va35	137	4.93
Mp04:126	Mp313E × Va35	247	5.51
Mp04:127	Mp313E × Va35	84	4.44
Mp313E[r]		147	5.00
Mp420[r]		538	6.29
Mp715[r]		179	5.19
Mp717[r]		209	5.35
GA209[s]		5850	8.67
SC212M[s]		9671	9.18
Va35[s]		1192	7.08
Mean			5.83
LSD (0.05)			1.29
CV			16

[a]Data were transformed (ln[y + 1]) before statistical analysis.
[b]Geometric means were calculated by converting transformed means to the original scale (ng/g).
[s]Susceptible.
[r]Resistant check.

at Tifton (Table 3) and in single crosses evaluated at Mississippi State (Table 5). Mp04:97 flowers approximately 14 days earlier than Mp715. The late maturity of Mp715 limits its usefulness in commercial breeding programs. Several of the selections from Mp313E × Va35 exhibited levels of resistance equal to that of Mp313E. The level of contamination for Mp04:110 was significantly lower than that of Mp313E. This line flowers approximately seven days earlier than Mp313E.

Future Directions

Identifying germplasm with resistance is a critical step in developing maize hybrids with resistance to aflatoxin contamination. The value of the germplasm will depend on how efficiently the resistance can be incorporated into commercially viable maize

hybrids: hybrids that are not only resistant to aflatoxin contamination but also have other desirable agronomic qualities. While conventional phenotypic selection was successfully used to transfer resistance from Mp313E or Mp715 into lines with better agronomic qualities, selection based on molecular markers may be more useful in developing aflatoxin-resistant hybrids. Brooks and colleagues (2005), in an analysis of an Mp313E × B73 $F_{2:3}$ mapping population, identified quantitative trait loci (QTL) on chromosomes 2 and 4 that, depending on the year, accounted for 15% to 36% of the phenotypic variation associated with aflatoxin accumulation. Other QTL with smaller effects were also found. The number and relatively small effects of genes associated with resistance to aflatoxin accumulation have limited the effectiveness of molecular marker–assisted selection in transferring resistance from Mp313E into other lines at Mississippi State. A research program at the University of Illinois has had some success in identifying QTL associated with resistance and in incorporating the QTL into commercial breeding lines (Clements and White, 2005). Currently, resistance from Tex6 and Mp313E is being transferred into FR1064 via molecular marker–assisted selection.

The search for new sources of resistance to aflatoxin accumulation will continue at Mississippi State, Tifton, and Raleigh. As new sources are identified and developed, efforts to identify QTL associated with resistance will continue. QTL that have larger effects that are stable over environments will be of particular interest. Identifying QTL associated with resistance to aflatoxin accumulation that are not associated with late maturity or tight husk coverage will also be useful in developing commercially viable maize hybrids. Association mapping techniques will also be used so that a large number of inbred lines can be analyzed (Yu and Buckler IV, 2006). This type of analysis should be helpful in determining which germplasm lines possess unique genes for resistance and which sources can be used together to increase overall resistance to aflatoxin contamination.

Acknowledgments

Appreciation is extended to Michael Blanco for his advice in choosing GEM selections for evaluation, and to Michael Clements, who conducted the initial evaluations of much of

the germplasm. This manuscript is a joint contributionof USDA-ARS and the Mississippi Agricultural Experiment Station. It is published as Journal No. J-11357 of the Mississippi Agricultural and Forestry Experiment Station.

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